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IN VITRO FREE RADICAL SCAVENGING ACTIVITY OF METHANOL EXTRACT OF THE LEAVES OF *MIMUSOPS ELENGI LINN*.

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ABSTRACT

In the present study, antioxidant potential of the methanol extract of the leaves of *Mimusops elengi* Linn. was evaluated by using 1, 1-diphenyl-2-picrylhydrazyl (DPPH) scavenging assay, reducing power and total antioxidant capacity. The extract showed significant activities in all antioxidant assays compared to the reference antioxidant ascorbic acid in a dose dependent manner. In DPPH scavenging assay the IC_{50} value of the extract was found to be 43.26µg/ml while the IC_{50} value of the reference standard ascorbic acid was 58.92 µg/ml. Total antioxidant activity was also found to increase in a dose dependent manner. Moreover, *M. elengi* extract showed strong reducing power. These results suggest that *Mimusops elengi* may act as a chemopreventative agent, providing antioxidant properties and offering effective protection from free radicals.

Key words: *Mimusops elengi*, antioxidant, reducing power, total antioxidant capacity, reactive oxygen species

INTRODUCTION

Antioxidant research is an important topic in the medical field as well as in the food industry. Recent research with important bioactive compounds in many plant and food materials have received much attention. The oxidation induced by ROS can result in cell membrane disintegration, membrane protein damage and DNA mutation, which can further initiate or propagate the development of many diseases, such as cancer, liver injury and cardiovascular disease (Liao and Yin, 2000). Although the body possesses such defense mechanisms, as enzymes and antioxidant nutrients, which arrest the damaging properties of ROS (Halliwell *et al*., 1995; Sies, 1993), continuous exposure to chemicals and contaminants may lead to an increase in the amount of free radicals in the body beyond its capacity to control them, and cause irreversible oxidative damage (Tseng *et al.,* 1997). Therefore, antioxidants with free radical scavenging activities may have great relevance in the prevention and therapeutics of diseases in which oxidants or free radicals are implicated (Soares *et al.,* 1997). In this respect, polyphenolic compounds, like flavonoids and phenolic acids, commonly found in plants have been reported to have multiple biological effects, including antioxidant activity (Brown and Rice-Evans, 1998; Gil *et al.,* 1999; Kähkönen *et al.,* 1999; Vinson *et al.,* 1995). Currently, the possible toxicity of synthetic antioxidants has been criticized. It is generally assumed that frequent consumption of plant-derived phytochemicals from vegetables, fruit, tea, and herbs may contribute to shift the balance toward an adequate antioxidant status (Halliwell, 1996). Thus interest in natural antioxidant, especially of plant origin, has greatly increased in recent years (Jayaprakash and Rao, 2000). *Mimusops elengi* Linn commonly known as Bakul belongs to the family *Sapotaceae* and is a small to large evergreen tree found all over the different parts of Bangladesh, Pakistan and India (Ghani, 2003). It is cultivated in gardens as an ornamental tree for sweet-scented flowers. It has been used in the indigenous system of medicine for the treatment of various ailments. Several therapeutic uses such as cardiotonic, alexipharmic, stomachic, anthelmintic and astringent have been ascribed to the bark of *Mimusops elengi* (Ghani, 2003; Kirtikar and Basu, 1935). The bark and fruit of this plant are used in the treatment of diarrhea and dysentery, and a decoction of the bark is used as a gargle (Ghani, 2003; Jahan *et al*., 1995). The pounded seeds pasted with oil are used for the treatment of obstinate constipation. Pillow stuffing made from the dried flowers induces nasal discharge and relieves headache (Jahan *et al.,* 1995). Several triterpenoids, steroids, steroidal glycosides, flavonoids, and alkaloids have been reported from this species (Jahan *et al.,*1995; Sahu *et al.,* 1997).Phytochemical review shows the presence of taraxerol, taraxerone, ursolic acid, (Misra and Mitra, 1967; Misra and Mitra, 1968) betulinic acid, V-spinosterol, W-sitosterol, lupeol (Misra and Mitra, 1967; Misra and Mitra, 1968), alkaloid isoretronecyl tiglate (Hart *et al.,* 1968) and mixture of triterpenoid saponins in the bark of *Mimusops elengi*.

As a part of our ongoing investigations on natural antioxidants from local medicinal plants of Bangladesh (Alam *et al.*, 2008a, Alam *et al*., 2008b, Saha *et al*., 2008.), in this paper, we reported a study of the antioxidant activity of the leaves of *M. elengi.* The evaluation of antioxidant power was performed in vitro by the DPPH, reducing power and total antioxidant capacity assays.

MATERIALS AND METHODS

Chemicals

DPPH (1, 1-diphenyl, 2-picrylhydrazyl), TCA (trichloroacetic acid) and ferric chloride were obtained from Sigma Chemical Co. USA. Ascorbic acid was obtained from SD Fine Chem. Ltd., Biosar, India. Ammonium molybdate was purchased from Merck, Germany.

Plant material

Leaves of *Mimusops elengi* were collected from Siddeswari campus, Stamford University, Bangladesh in June 2007, and identified by Professor Dr. Abdul Ghani (Stamford University, Dhaka, Bangladesh); a voucher specimen (SU-MAA-2007-3) for this collection has been retained in the Pharmacognosy Laboratory, Stamford University, Dhaka, Bangladesh.

Extraction

The shade-dried leaves were coarsely powdered and extracted with 95% methanol by a Soxhlet apparatus at 45^{o}C. The solvent was completely removed by rotary evaporator and obtained greenish gummy exudates. This crude extract was used for further investigation for potential antioxidant properties.

Antioxidant activity test

DPPH radical scavenging activity

Qualitative analysis

The methanol extract was applied on a TLC plate as a spot (100 µg/ml) for chromatographic separation of the extract using the mobile phase methanol:chloroform (95:5, v/v). It was allowed to develop the chromatogram for 30 minutes. After completion of the chromatogram the whole plate was sprayed with DPPH (0.15 % w/v) solution using an atomizer. The color changes (yellowish color development on pinkish background on the TLC plate) were noted as an indicator of the presence of antioxidant substances.

Quantitative analysis

The free radical scavenging capacity of the extracts was determined using DPPH (Braca *et al.*, 2001). DPPH solution (0.004% w/v) was prepared in 95% methanol. Metahnol extract of *Mimusops elengi was* mixed with 95 % methanol to prepare the stock solution (5 mg/mL). Freshly prepared DPPH solution (0.004% w/v) was taken in test tubes and *Mimusops elengi* extracts was added followed by serial dilutions (1 µg to 500 µg) to every test tube so that the final volume was 3 mL and after 10 min, the absorbance was read at 515 nm using a spectrophotometer (HACH 4000 DU UV – visible spectrophotometer). Ascorbic acid was used as a reference standard and dissolve in distilled water to make the stock solution with the same concentration (5 mg/mL). Control sample was prepared containing the same volume without any extract and reference ascorbic acid. 95 % methanol was served as blank. % scavenging of the DPPH free radical was measured by using the following equation:

$$\%\text{Scavenging Activity} = \frac{\text{Absorbanc of the control} - \text{Absorbance of the test sample}}{\text{Absorbance of the control}} \times 100$$

The inhibition curve was plotted for duplicate experiments and represented as % of mean inhibition ± standard deviation. IC_{50} values were obtained by probit analysis. (Viturro *et al*., 1999).

Determination of total antioxidant capacity

The antioxidant activity of the extract was evaluated by the phosphomolybdenum method according to the procedure describe by Prieto *et al*. (1999). The assay is based on the reduction of Mo (VI)–Mo (V) by the extract and subsequent formation of a green phosphate/Mo (V) complex at acid pH. A 0.3 ml extract was combined with 3 ml of reagent solution (0.6 M sulfuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate). The tubes containing the reaction solution were incubated at 95^0C for 90 min. Then the absorbance of the solution was measured at 695 nm using a spectrophotometer (HACH 4000 DU UV – visible spectrophotometer) against blank after cooling to room temperature. Methanol (0.3 ml) in the place of extract is used as the blank. The antioxidant activity is expressed as the number of gram equivalents of ascorbic acid.

Reducing power

The reducing power of *Mimusops elengi* was determined according to the method previously described by Oyaizu (Oyaizu, 1986). Different concentrations of *Mimusops elengi* extract (100– 1000 µg) in 1 ml of distilled water were mixed with phosphate buffer (2.5 ml, 0.2 M, pH 6.6) and potassium ferricyanide [$K_3Fe(CN)_6$] (2.5 ml, 1%). The mixture was incubated at 50°C for 20 min. A portion (2.5 ml) of trichloroacetic acid (10%) was added to the mixture, which was then centrifuged at 3000 rpm for 10 min. The upper layer of the solution (2.5 ml) was mixed with distilled water (2.5 ml) and $FeCl_3$ (0.5 ml. 0.1%) and the absorbance was measured at 700 nm. Increased absorbance of the reaction mixture indicated increased reducing power. Ascorbic acid was used as the standard. Phosphate buffer (pH 6.6) was used as blank solution. The absorbance of the final reaction mixture of two parallel experiments was taken and is expressed as mean ± standard deviation.

RESULTS AND DISCUSSION

In traditional societies nutrition and health care are strongly interconnected and many plants have been consumed both as food and for medicinal purposes. The consumption of non-cultivated botanicals plays a central role in the diet, but very few ethnopharmacological and phytopharmacological studies have dealt exhaustively with the potential health benefits of such diets

In the past few years, there has been growing interest in the involvement of reactive oxygen species (ROS) in several pathological situations. ROS produced in vivo include superoxide radical ($O_2^{\bullet-}$), hydrogen peroxide (H_2O_2) and hypochlorous acid (HOCl). H_2O_2 and $O_2^{\bullet-}$ can interact in the presence of certain transition metal ions to yield a highly-reactive oxidizing species, the hydroxyl radical ($^{\bullet}OH$) (Aruoma and Halliwell, 1987). Phenolic compounds and flavonoids have been reported to be associated with antioxidative action in biological systems, acting as scavengers of singlet oxygen and free radicals (Rice-Evans *et al*., 1997; Jorgensen *et al*., 1999). Literature reviews confirm that triterpenoids, steroids, steroidal glycosides, flavonoids, and alkaloids are contained in this species (Jahan *et al*., 1995). Many naturally occurring triterpinoids exhibited a good anti-inflammatory activity have been isolated from various plants (Fernandez *et al*., 2001; Ismaili *et al*., 2002). Pentacyclic triterpinoids have a wide spectrum of biological activities and some of them may be useful in medicine. There is growing interest in natural triterpinoids caused as much by the scientific aspects extraction and structural analysis of these compounds, as by the fact of their wide spectrum of biological activities, they are bactericidal, fungicidal, antiviral, cytotoxic, analgesic, antiinflammatory, anti-cancer and antiallergic (Patocka , 2003).

The DPPH (1,1-diphenyl-2-picrylhydrazyl) radical scavenging activity of *M. elengi* is given in Fig. 1. This activity was increased by increasing the concentration of the sample extract. DPPH antioxidant assay is based on the ability of 1,1-diphenyl-2-picryl-hydrazyl (DPPH), a stable free radical, to decolorize in the presence of antioxidants. The DPPH radical contains an odd electron, which is responsible for the absorbance at 515 nm and also for a visible deep purple color. When DPPH accepts an electron donated by an antioxidant compound, the DPPH is decolorized, which can be quantitatively measured from the changes in absorbance. The IC_{50} value of the extract was 43.26µg/ml, as opposed to that of ascorbic acid (IC_{50} 55.89 µg/mL), which is a well known antioxidant.

Total antioxidant capacity of the *Mimusops elengi* extract, expressed as the number of gram equivalents of ascorbic acid, is shown in Table 1. The phosphomolybdenum method was based on the reduction of Mo (VI) to Mo (V) by the antioxidant compound and the formation of a green phosphate/Mo (V) complex with a maximal absorption at 695 nm.

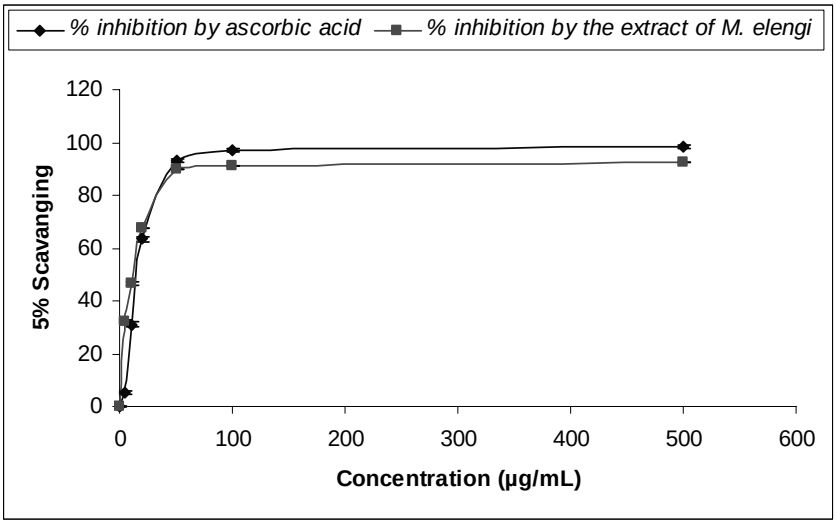

Fig. 1. DPPH radical scavenging activity of the methanol extract *Mimusops elengi.* Values are the average of duplicate experiments and represented as mean± standard deviation.

Table 1. Total antioxidant capacity of the plant extracts *Mimusops elengi*

Materials	Concentration (µg/mL)	Equivalent to ascorbic acid
Methanol extract of *Mimusops elengi*	100	0.33±0.15
	200	1.37±0.07
	400	1.49±0.15
	600	2.61±0.14
	800	3.68±0.14

As mentioned above, these plants contain ursolic acid, betulinic acid and terpenoids. The antioxidative effect is mainly due to phenolic components, such as phenolic acids, and phenolic diterpenes (Shahidi *et al.*, 1992). The antioxidant activity of phenolic compounds is mainly due to their redox properties, which can play an important role in absorbing and neutralizing free radicals, quenching singlet and triplet oxygen, or decomposing peroxides (Osawa, 1994). For the measurements of the reductive ability, it has been found that the Fe^{3+}–Fe^{2+} transformation occurred in the presence of extract samples which was postulated previously by Oyaizu (1986).

Earlier authors (Tanaka *et al.,* 1988) have observed a direct correlation between antioxidant activity and reducing power of certain plant extracts. The reducing properties are generally associated with the presence of reductones (Duh *et al*., 1999), which have been shown to exert antioxidant action by breaking the free radical chain by donating a hydrogen atom (Gordon, 1990). Reductones are also reported to react with certain precursors of peroxide, thus preventing peroxide formation. Figure 2 shows the reductive capabilities of the plant extract compared to ascorbic acid. The reducing power of extract of *Mimusops elengi* was found remarkable and the reducing power of the extract was observed to rise as the concentration of the extract gradually increased.

From the above results and discussion it can be concluded that the methanol extract of *Mimusops elengi* possesses the potent antioxidant substances which may be responsible for its anti-inflammatory and chemoprotective mechanism as well as justify the basis of using this plant's extract as folkloric remedies.

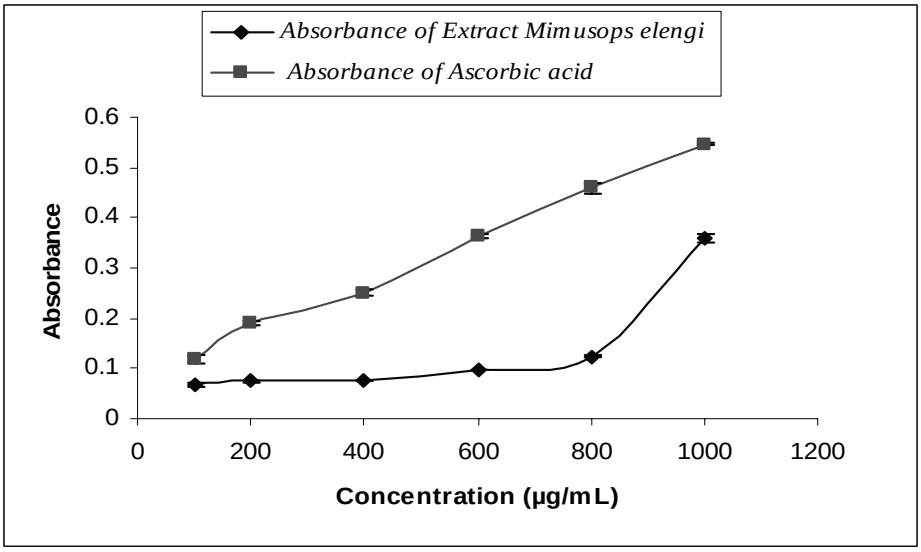

Fig. 2. Reducing power of the crude plant extract *Mimusops elengi*. Values are the average of duplicate experiments and represented as mean± standard deviation.

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